

Pigmentation and Delayed Oxidation of Broiler Chickens by the Red Carotenoid, Astaxanthin, from Chemical Synthesis and the Yeast, *Xanthophyllomyces dendrorhous**

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ABSTRACT : The red carotenoid, astaxanthin was studied to improve the meat quality of broiler chickens. Astaxanthin pigmented chickens and delayed oxidation of lipid in them. Two sources of astaxanthin were used to pigment broiler chickens in a five-wk feeding trial: biological astaxanthin (BA) from the red yeast, *Xanthophyllomyces dendrorhous*, and chemical astaxanthin (CA) from chemical synthesis. The concentrations of CA (45 mg/kg feed) and BA (22.5 mg/kg feed) were set to give similar levels of pigmentation. The colorimetric values (a and b) of breast muscles were significantly changed by astaxanthin ($p \leq 0.01$). Absorption and accumulation of BA were higher than those of CA, probably due to the high contents of lipids in the yeast (17%). Lipid peroxide formation in skin was significantly decreased by astaxanthin ($p \leq 0.05$). This result indicated that the production of lipid peroxides in the carcasses of broiler chickens during storage could be delayed by astaxanthin. Therefore, astaxanthin could be used as an antioxidant as well as a colorant for broiler chickens. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 9 : 1309-1314)

Key Words : Antioxidant, Astaxanthin, Chicken, Pigmentation, *Xanthophyllomyces dendrorhous*

INTRODUCTION

Color of chickens greatly affects the purchasing behavior of consumers (Han et al., 1987; Fletcher, 1999). Corn, a usual ingredient of chicken feed, is the major source of carotenoids pigmenting egg-yolks and bodies. Poultry accumulate carotenoids mainly in liver, skin, and shank (Allen, 1988). Since carotenoids are not produced by chickens, they must be supplied in feed for pigmentation (Dua et al., 1967).

Astaxanthin accumulates in fish, poultry and crustaceans (Johnson et al., 1978, 1980). Recently, an interest in astaxanthin has been increased, because it also has a strong antioxidant effect (Miki, 1991). Akiba et al. (2001) observed that astaxanthin was successfully accumulated in the fatty tissues of chicken. Accumulated astaxanthin may increase the quality of chicken by improving flavor (Josephson, 1987), delaying oxidation, and pigmenting bodies.

Chemically synthesized astaxanthin (CA), produced by Hoffman La Roche (Switzerland) and BASF (Germany),

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has been used for pigmentation of aquacultured animals. Biological astaxanthin (BA) produced by the red yeast, *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*), successfully pigmented egg-yolks, bodies, and skins of laying hens and broiler chickens (Kim et al., 1996; Akiba et al., 2001). The cells of *X. dendrorhous* are composed of 5.6% ash, 40.3% carbohydrate, 30.1% protein, 8.2% RNA, 17.0% lipid, and 0.06-0.3% astaxanthin (Johnson et al., 1980; Johnson and An, 1991). Astaxanthin in the yeast has become bio-available to laying hens by HCl treatment and spray-drying after neutralization (Kim et al., 1996). Astaxanthin from *X. dendrorhous* was mainly (3R,3'R)-astaxanthin (Andrewes and Starr, 1976). The configuration ratio of synthetic astaxanthin was (3R,3'R):meso:(3S,3'S) = 1:2:1 (The catalog for Carophyll Pink, Hoffman La Roche, Switzerland). In this study, pigmentation and prevention of oxidation by two sources of astaxanthin in broiler chickens were examined.

MATERIALS AND METHODS

Birds and housing

D-old 240 male Ross broilers were randomly allotted to 24 raised wire-floor cages (100×72×50 cm). Temperature was maintained at 32-33°C up to Day 7 and gradually decreased to 22°C at wk 5. A continuous lighting was used for the first 3 d, and 16 h of light and 8 h of darkness were applied until the end of the 5 wk feeding trial.

Diets and design

Three experimental dietary treatments with eight cages

Table 1. Composition of experimental diets for broiler chickens*

Ingredients	Broiler starter (0-3 wk)	Broiler finisher (4-8 wk)		
		Control	BA ¹	CA ²
		----- (%) -----		
Ground corn	59.26	64.82	64.82	64.76
Soybean meal (44%)	20.52	18.92	13.92	18.92
Corn gluten meal (60%)	5.00	4.00	4.00	4.00
Fish meal (60%)	4.43	3.17	5.17	3.17
Rapeseed meal	5.00	3.00	3.00	3.00
Soybean oil	2.00	3.00	3.00	3.00
Calcium phosphate	1.78	1.08	1.08	1.08
Limestone	0.88	1.08	1.08	1.08
Salt	0.40	0.40	0.40	0.40
Biological astaxanthin ¹	0.00	0.00	3.00	0.00
Chemical astaxanthin ²	0.00	0.00	0.00	0.06
DL-methionine (50%)	0.34	0.16	0.16	0.16
L-lysine-HCl	0.19	0.17	0.17	0.17
Vit.-min. premix ³	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Calculated composition				
ME (kcal/kg)	3,100.00	3,200.00	3,200.00	3,200.00
Crude fat (%)	5.31	6.30	6.94	6.30
CP (%)	21.50	19.00	18.00	19.00
Ca (%)	1.00	0.90	0.90	0.90
Non phytate P (%)	0.45	0.35	0.35	0.35
Methionine (%)	0.50	0.50	0.50	0.50
Lysine (%)	1.10	1.00	1.00	1.00
Astaxanthin (mg/kg)	0.00	0.00	22.50	45.00

* D-old broilers grew with the broiler starter for 3 wk. Then the chickens were used for the 5 wk feeding trial with the broiler finisher. The initial average weight of the chickens was 1,049±32 g at the age of 3 wk.

¹ Biological astaxanthin: the red yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*).

² Chemical astaxanthin: Carophyll Pink, commercial colorant from Hoffman La Roche (Switzerland).

³ Provided followings per kg of diet: vit. A, 5,500 IU; vit. D₃, 1,100 ICU; vit. E, 11 IU; vit. B₁₂, 6.6 g; riboflavin, 4.4 mg; pantothenic acid, 11 mg; choline, 191 mg; menadione, 1.1 mg; folic acid, 0.55 mg; pyridoxine, 2.2 mg; biotin, 0.11 mg; thiamin, 2.2 mg; ethoxyquin, 125 mg; Mn, 120 mg; Zn, 100 mg; Fe, 60 mg; Cu, 10 mg; I, 0.46 mg.

(10 birds per cage) per treatment were used in a completely randomized design. One bird per cage was randomly selected for analysis and thus 8 replicates per treatment were designed.

The broiler starter (Table 1) was used for 3 wk to grow D-old broilers. Three-wk-old chickens grown with the broiler starter were used for the 5 wk feeding trial. CA diet was prepared by adding 0.56 g of Carophyll Pink (astaxanthin 8%, w/w, Hoffman La Roche, Switzerland) per kg control finisher diet to give 45 mg/kg of astaxanthin (Table 1). BA diet was prepared by replacing soybean meal (5%) with fish meal (2%) and *X. dendrorhous* (3%), to give 22.5 mg/kg of astaxanthin (Table 1).

Feed and water were provided *ad libitum*. ME was controlled to be about 3,000 kcal/kg feed (Ru et al., 2003). Feed consumption and BW were recorded at the beginning and end of the 5 wk feeding trial with the broiler finisher.

Biological astaxanthin preparation

For the production of biological astaxanthin, *X. dendrorhous* strain 2A2N (An et al., 1996) was used in this study. The media and culture conditions used in this study

were previously described (An et al., 1996; An, 2001). Yeast cells were harvested and washed by a continuous centrifuge (50 L/h). The harvested yeasts were treated with HCl (2 M) at 50-90°C in a 50 L reactor. Yeast cells were finally dried by a spray-dryer at 150°C of inlet and at 85°C of outlet temperature. The whole yeast was used.

Blood and carcass preparation

At the termination of the feeding trial, one bird from each pen was randomly selected and blood was obtained from the wing vein. To 1 mL of EDTA-treated whole blood, 2 mL of dimethyl sulfoxide, 2 mL of acetone, 1 mL of petroleum ether, and 2 mL of 20% NaCl were serially added on vortex. After centrifugation (3,000 rpm, 3 min), the upper petroleum ether layer was filtered and used for HPLC analysis. After blood sampling, these birds were slaughtered for various carcass analyses. Skin for carotenoid analysis was roughly cut with scissors and homogenized (Polytron PT-MR2100, Kinematica Co., Switzerland). The breast meat was kept in a refrigerator (4°C) for 24 h for future analysis.

Table 2. Weight gain and feed intake of the broiler chickens

	Weight gain (g/bird)	Feed intake (g/bird)	Gain/feed
Control	1,366±115*	3,220±197	0.424±0.226
Biological astaxanthin ¹	1,406±137	3,226±186	0.446±0.304
Chemical astaxanthin ²	1,397±51	3,148±166	0.434±0.225

* Mean±SD obtained from 8 replicates.

¹ Biological astaxanthin; the red yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*).

² Chemical astaxanthin; Carophyll Pink, commercial colorant from Hoffman La Roche (Switzerland).

Carotenoid analysis

Carotenoid extraction from *X. dendrorhous* was previously described (An et al., 1996). The process for the extraction of carotenoids from 1 g of the homogenized skin was the same as that from 1 mL of blood. Carotenoid obtained from the skin contained high contents of lipids and thus the extracts were pretreated as follows. The petroleum ether layer was loaded on silica thin layer chromatography plate. The carotenoid containing silica was scraped and eluted with acetone. The acetone was filtered and used for HPLC analysis.

The carotenoid extracts were analyzed by an HPLC (Younglin Instrument Co., Seoul, Korea). The carotenoid extract (20 µl) was injected into Nucleosil column (100 Å; MetaChem Technologies Inc., Torrance, CA, USA) and carotenoids were detected by a UV-visible detector at 476 nm. Mobile system was *t*-butyl methyl ether:hexane:isopropanol:methanol=30:65:2.5:2.5 and the flow rate was 1.5 mL/min. For the quantification of carotenoids, β-carotene (95%, w/w; Sigma catalog # C-9750), lutein (70%, w/w; Sigma catalog # A-6250), and astaxanthin (98%, w/w; Sigma catalog # A-9335) were used as standards.

Colorimetric analyses

The breast meat was removed from the refrigerator and was exposed to room temperature for 30 min and cut into 35×25×6 mm. The Lightness (*L*), redness (*a*) and yellowness (*b*) values were determined by a colorimeter (CR-200, Minolta, Osaka, Japan).

Antioxidant effect of astaxanthin in skin

The antioxidant activity of astaxanthin in skin was measured by measuring aldehyde formation during storage (Kosugi et al., 1989). Skin of chickens was incubated at 30°C with continuous lighting for 0-8 days. Aldehydes were detected by using 2-thiobarbituric acid (TBA) and malonaldehyde was used as a standard. Skin samples of 2.4 g were added into 6 mL of 20% trichloroacetic acid (TCA) solution (20 g TCA in 100 mL of 2 M H₃PO₄ at 4°C) and mixed for 5 min. Distilled water was added to the skin-TCA solution mixture to be 12.5 mL of total volume. The diluted

Table 3. Colorimetric values of the broiler breast muscles

	Lightness (<i>L</i>)	Redness (<i>a</i>)	Yellowness (<i>b</i>)
Control	46.43±3.68*	-0.65±0.85 ^b	4.78±1.33 ^b
Biological astaxanthin ¹	44.74±5.49	2.61±1.17 ^a	8.47±1.66 ^a
Chemical astaxanthin ²	46.50±2.21	2.51±0.91 ^a	10.15±1.05 ^a

* Mean±SD obtained from 8 replicates.

^{a, b} Means within a column with different superscripts differ significantly ($p \leq 0.01$).

¹ Biological astaxanthin; the red yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*).

² Chemical astaxanthin; Carophyll Pink, commercial colorant from Hoffman La Roche (Switzerland).

mixture was passed through Whatman No. 1 filter paper. The filtered solution 3 mL was mixed with 3 mL of 5 mM TBA solution. The mixture was kept for 15 h without light. Absorbance of the solution was measured at 530 nm.

Statistical analysis

All data were subjected to one-way ANOVA. When the *F*-value was significant ($p \leq 0.05$ or $p \leq 0.01$), post-ANOVA test was conducted by using Tukey's test.

RESULTS AND DISCUSSION

Decision of biological and chemical astaxanthin levels

Our group previously reported that astaxanthin from *X. dendrorhous* at 20 and 30 mg/kg feed was accumulated in egg-yolks but poorly at 5 and 10 mg/kg feed (Kim et al., 1996). However, the high level (30 mg/kg) gave a decreased efficiency of astaxanthin accumulation compared to 5-10 mg/kg feed. Akiba et al. (2001) reported that BA at 15, 20, and 30 mg/kg feed significantly affected the colorimetric *a* (redness) values of various parts of broiler chickens. Therefore, the BA level at 22.5 mg/kg feed was used in this study, which was the mid-point of the proper pigmentation range from 15 to 30 mg/kg feed.

To decide the level of CA, a pre-experiment was performed and the result indicated that CA at levels twice that of BA gave a similar pigmentation of skin (data not shown but refer to Figure 3). Therefore, CA at 45 mg/kg feed was used in this study.

Effect of astaxanthin on growth performance

The growth rate and feed intake of broiler chickens were not affected ($p > 0.05$) by astaxanthin feeding (Table 2). However, although not significant, the gain/feed ratio was highest in the red yeast treatment (BA). It was well informed the good nutritional quality of the yeast (Johnson and An, 1991).

Pigmentation by astaxanthin

Astaxanthin affected the color of muscle as well as



Figure 1. Pigmentation of the broiler chickens by the 5-wk feeding of astaxanthin. Abbreviations: BA, Biological astaxanthin, 22.5 mg astaxanthin/kg feed (dried cells of the red yeast, *Xanthophyllomyces dendrorhose*); CA, Chemical astaxanthin, 45 mg astaxanthin/kg feed (Carophyll Pink[®] purchased from Hofman La Roche).

Table 4. Ratios of astaxanthin contents in blood, feed and skin

	Blood/feed ([ng/ml blood]/ [mg/kg feed])	Skin/feed ([ng/g skin]/ [mg/kg feed])	Skin/blood ([ng/g skin]/ [ng/ml blood])
Biological astaxanthin	43	66	1.54
Chemical astaxanthin	23	28	1.20

Data were obtained by calculating data from Figure 1 and 2.

lipid-rich parts (skin and lipid components) (Figure 1). It gave orange-red color to the broiler chickens (Table 3). Lightness (*L* value) was not significantly affected by astaxanthin but redness (*a* values) and yellowness (*b* values) were significantly increased ($p \leq 0.01$). The broiler chickens fed BA (22.5 mg/kg of astaxanthin) and CA (45 mg/kg of astaxanthin) did not show significant differences in the color of muscles from each other. Even though the content of astaxanthin in feeds was different, the levels of pigmentation in breast muscles were similar. Therefore, absorption of carotenoids into blood and accumulation on skin were measured.

Astaxanthin contents in blood of the chickens fed with BA and CA were 860 and 1,050 ng/ml blood, respectively (Figure 2). The ratios of astaxanthin in blood (ng/mL) to that in feed (mg/kg) were 43 in the case of BA and 23 in the case of CA (Table 4), indicating that absorption efficiency

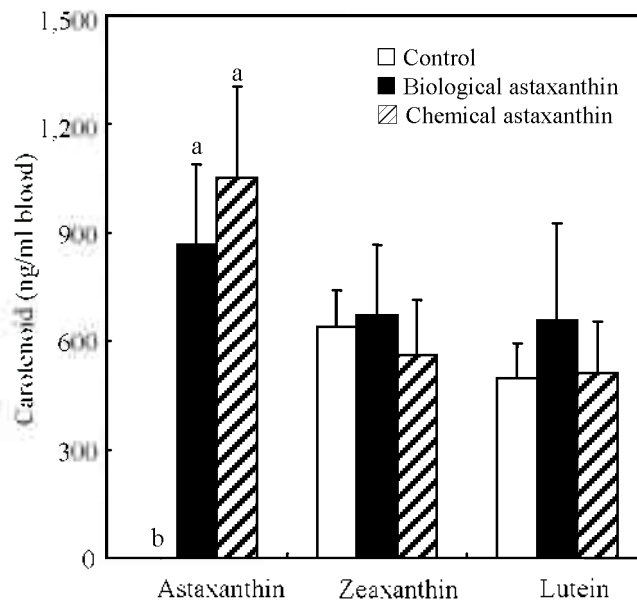


Figure 2. Carotenoid contents in blood of the broiler chickens. ^{a, b} Within a variable, bars lacking common letters differ significantly ($p \leq 0.01$). Abbreviations: BA, Biological astaxanthin (dried cells of the red yeast, *Xanthophyllomyces dendrorhose*); CA, Chemical astaxanthin (Carophyll Pink[®] purchased from Hofman La Roche).

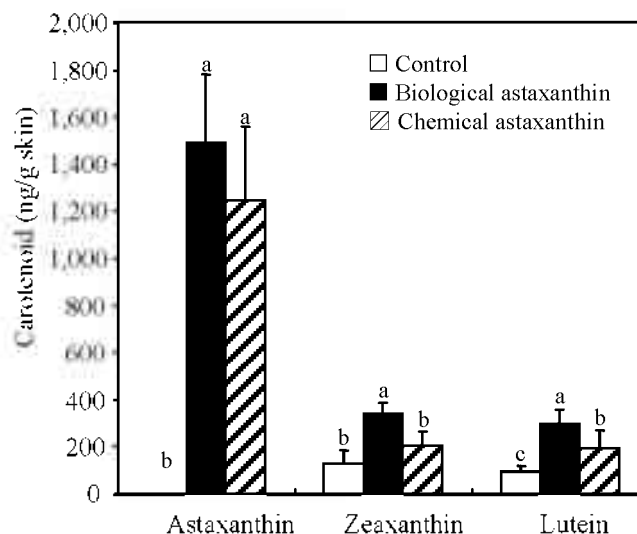


Figure 3. Carotenoid contents in skin of the broiler chickens after the 5-wk feeding. ^{a, b} Within a variable, bars lacking common letters differ significantly ($p \leq 0.05$). Abbreviations: BA, Biological astaxanthin (dried cells of the red yeast, *Xanthophyllomyces dendrorhose*); CA, Chemical astaxanthin (Carophyll Pink[®] purchased from Hofman La Roche).

of BA was higher than that of CA. Astaxanthin contents in skin of the chickens fed with BA and CA were 1,490 and 1,240 ng/g skin, respectively (Figure 3). The ratios of astaxanthin in skin to blood of BA and CA were 1.54 and 1.2, respectively (Table 4). These data indicate that carotenoid transport efficiency of BA from blood to skin

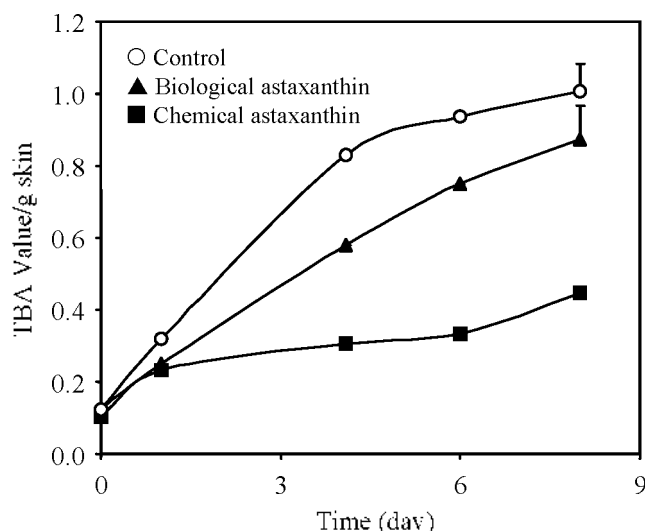


Figure 4. Antioxidant activity of astaxanthin in skin. Bars indicate S.D. Abbreviations: BA, Biological astaxanthin (dried cells of the red yeast, *Xanthophyllomyces dendrorhous*); CA, Chemical astaxanthin (Carophyll Pink[®] purchased from Hofman La Roche).

was also higher than that of CA.

Since carotenoids are non-polar, they should be absorbed along with lipids in the small intestine. The high contents of lipids in *X. dendrorhous* (0.51% increased by the yeast) probably caused an increased utilization of astaxanthin, compared to chemical astaxanthin. Similar observations were made by Day and Williams (1958), Han et al. (1987) and Jayarajan et al. (1980). It is of interest to note that the contents of the major carotenoids of corns, i.e., lutein and zeaxanthin, in blood and skin of the chickens fed BA were higher than in the chickens fed CA (Figures 2 and 3), probably due to the high contents of lipids in BA.

Delayed peroxidation of lipid by astaxanthin during storage of carcass

The antioxidant activity of astaxanthin in the skin was measured by accelerating the oxidation of skin lipid. Skin was homogenized and incubated at 30°C under illumination. Production of lipid peroxide during storage was notably decreased by astaxanthin (Figure 4). The peroxide production in chickens fed CA was markedly lowered compared to BA (Figure 4). The BA from *X. dendrorhous* was mainly (3R,3'R)-astaxanthin (Andrewes and Starr, 1976). The configuration ratio of CA was (3R,3'R):meso:(3S,3'S)=1:2:1 (The catalog for Carophyll Pink, Hoffman La Roche, Switzerland). Probably, meso- or (3S,3'S)-astaxanthin might have a strong antioxidant activity, compared to (3R,3'R)-astaxanthin.

In this study, astaxanthins, from the red yeast *X. dendrorhous* and chemical synthesis, pigmented the skin of the broiler chickens. At the TBA points higher than 0.4, chicken skin emitted unacceptable off-flavor. Astaxanthin in

the skin inhibited lipid peroxidation and delayed the production of off-flavor. Antioxidant activity of astaxanthin was 10 times higher than that of β -carotene and 100 times higher than that of tocopherol (Miki, 1991). Conclusively, astaxanthin can be used to improve the quality of broiler chickens

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